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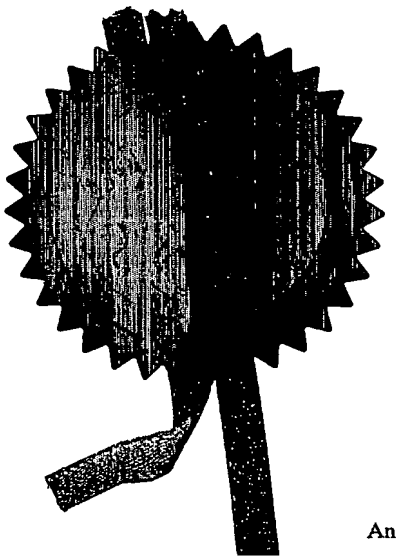

Signed P. Mahoney

Dated 19 January 2004

Patents Form 1/77

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1/77

10DEC02 E769773-1 001631
P01/7700 0.00-0228723.3

Request for grant of a patent

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Fee: £0

1. Your reference — 45481.GB01/JMD/NT

2. Patent application number
(The Patent Office will fill in this part) — 0228723.3

3. Full name, address and postcode of the or of each applicant *(underline all surnames)*

Cambridge Biotechnology Ltd
P.O.Box 230
Cambridge CB2 1XJ

Patents ADP number *(if you know it)* — 8208761002

If the applicant is a corporate body, give the country/state of incorporation — United Kingdom

4. Title of the invention

Treatment of Pain

5. Full name, address and postcode in the United Kingdom to which all correspondence relating to this form and translation should be sent

Reddie & Grose
16 Theobalds Road
LONDON
WC1X 8PL

Patents ADP number *(if you know it)* — 91001

6. If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or of each of these earlier applications and *(if you know it)* the or each application number

Country	Priority application *(If you know it)*	Date of filing *(day/month/year)*

7. If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application

Number of earlier application	Date of filing *(day/month/year)*

8. Is a statement of inventorship and of right to grant of a patent required in support of this request? *(Answer 'Yes' if:*
a) any applicant named in part 3 is not an inventor, or
b) there is an inventor who is not named as an applicant, or
c) any named applicant is a corporate body.
See note (d))

YES

Patents Form 1/77

9. Enter the number of sheets for any of the following items you are filing with this form. Do not count copies of the same document.

Continuation sheets of this form	
Description	5
Claim(s)	2
Abstract	0
Drawing(s)	3 + 3

10. If you are also filing any of the following, state how many against each item.

Priority documents	0
Translations of priority documents	0
Statement of inventorship and right to grant of a patent *(Patents Form 7/77)*	0
Request for preliminary examination and search *(Patents Form 9/77)*	0
Request for substantive examination *(Patents Form 10/77)*	0
Any other documents *(please specify)*	0

11. I/We request the grant of a patent on the basis of this application.

Signature: Reddie e Grose

Date: 9 December 2002

12. Name and daytime telephone number of person to contact in the United Kingdom: J M DAVIES 01223-360350

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Treatment of Pain

This invention relates to an anti-hyperalgesic and to methods of preventing, treating, or ameliorating hyperalgesia using the anti-hyperalgesic.

Hyperalgesia is a condition of heightened pain perception caused by tissue damage. This condition is a natural reponse of the nervous system apparently designed to encourage protection of the damaged tissue by an injured individual, to give time for tissue repair to occur. There are two known underlying causes of this condition, an increase in sensory neuron acitivity, and a change in neuronal processing of nociceptive information which occurs in the spinal cord. Hyperalgesia can be debilitating in conditions of chronic inflammation (e.g. rheumatoid arthritis), and when sensory nerve damage has occurred (i.e. neuropathic pain).

Two major classes of analgesics are known: (i) non steroidal anti-inflammatory drugs (NSAIDs) and the related COX-2 inhibitors; and (ii) opiates based on morphine. Analgesics of both classes are effective in controlling normal nociceptive pain. However, they are less effective against some types of hyperalgesic pain, such as neuropathic pain. Many medical practitioners are reluctant to prescribe opiates at the high doses required to affect neuropathic pain because of the side effects caused by administration of these compounds, and the possibility that patients may become addicted to them. NSAIDs are much less potent than opiates, so even higher doses of these compounds are required. However, this is undesirable because these compounds cause irritation of the gastro-intestinal tract.

Adenosine A1 receptor agonists are known to act as powerful analgesics (Sawynok, Eur J Pharmacol. (1998) 347, 1-11), and adenosine A2A receptor agonists are known to act as anti-inflammatory agents. However, development of adenosine-based therapies has generally been precluded because they have unacceptable side effects. Selective A1 receptor agonists cause bradycardia, and A2A receptor agonists cause widespread vasodilation with consequent hypotension and tachycardia.

There is, therefore, a need to provide anti-hyperalgesics which are sufficiently potent to control pain perception in neuropathic and other hyperalgesic syndromes, and which do not have serious side effects or cause patients to become addicted to them.

Spongosine is a compound that was first isolated from the tropical marine sponge, *Cryptotethia crypta* in 1945 (Bergmann and Feeney, J. Org. Chem. (1951) 16, 981, Ibid (1956) 21, 226). Spongosine was the first methoxypurine found in nature, and is also known as 2-methoxyadenosine, or 9H-purin-6-amine, 9-α-D-arabinofuranosyl-2-methoxy.

The first biological activities of spongosine were described by Bartlett *et al.* (J. Med. Chem. (1981) 24, 947-954) who showed that this compound has muscle relaxant, hypothermic, hypotensive, and anti-inflammatory activity in rats.

The affinity of spongosine for the rat adenosine A1 and A2A receptors has been determined. The Kd values obtained were 340nM for the A1 receptor and 1.4μM for the A2A receptor (Daly *et al.*, Pharmacol. (1993) 46, 91-100). In the guinea pig, the efficacy of spongosine was tested in the isolated heart preparation and the EC50 values obtained were 10 μM and 0.7 μM for the adenosine A1 and A2A receptors, respectively (Ueeda et al J Med Chem (1991) 34, 1334-1339). In the early 1990s the other adenosine receptors (the A2B and A3 receptors) were cloned, but the activity of spongosine at these receptors was never investigated. The low potency and poor receptor selectivity of this compound led to it being largely ignored as more and more potent and receptor selective novel compounds were synthesised.

It has surprisingly been found that spongosine when administered to mammals gives significant pain relief in conditions of increased pain sensitivity (such as neuropathic and inflammatory hyperalgesia), without causing the significant side effects expected from use of purine receptor agonists.

According to the invention there is provided use of spongosine in the manufacture of a medicament for the prevention, treatment, or amelioration of hyperalgesia.

There is also provided according to the invention a method of preventing, treating, or ameliorating hyperalgesia which comprises administering spongosine to a subject in need of such prevention, treatment, or amelioration.

Spongosine has been found to be effective in inhibiting pain perception in mammals suffering from neuropathic and inflammatory pain even when administered at doses expected to give concentrations well below those known to activate adenosine receptors. Thus, spongosine can treat neuropathic and inflammatory pain without causing the significant side effects associated with administration of other adenosine receptor agonists.

No anti-inflammatory effects were observed after administration of spongosine, nor was any analgesic effect on normal physiological nociception observed.

Spongosine can be used as an anti-hyperalgesic for the prevention, treatment, or amelioration of hyperalgesia caused as a result of neuropathy, including bowel pain, back pain, cancer pain, HIV pain, phantom limb pain, post-operative pain, diabetic neuropathy, polyneuropathy, post-herpes neuralgia, and trigeminal neuralgia.

Spongosine can be used as an anti-hyperalgesic for the prevention, treatment, or amelioration of hyperalgesia caused as a result of inflammatory disease, including bowel pain, back pain, cancer pain, fibromyalgia, post-operative pain, osteoarthritis, and rheumatoid arthritis.

It will be appreciated that spongosine may be administered together with a pharmaceutically acceptable carrier, excipient, or diluent.

The appropriate dosage of spongosine will vary with the age, sex, and weight of the subject being treated, and the route of administration.

It is preferred that spongosine is administered at a dose that is one fifth to one fiftieth, preferably one fifth to one tenth, of the minimum dose of spongosine that gives rise to bradycardia, hypotension or tachycardia side effects in animals of the same species as the subject to which the dose is to be administered.

Preferably spongosine is administered at a dose of less than 6mg/kg, and preferably at least 0.1mg/kg. More preferably spongosine is administered at a dose of 0.2 to 1mg/kg.

Spongosine may be administered by any suitable route, preferably orally, parenterally, sublingually, transdermally, intrathecally, or transmucosally.

Preferably spongosine is administered at a frequency of 2 or 3 times per day.

Embodiments of the invention are described in the following examples with reference to the accompanying drawings in which:

Figure 1 shows the anti-hyperalgesic actions of spongosine (0.6 mg/kg p.o.) on carrageenan induced hyperalgesia. A: time course; B: dose dependency of the anti-hyperalgesic effect;
Figure 2 shows the anti-hyperalgesic actions of spongosine (0.6 mg/kg p.o.) in the chronic constriction injury model of neuropathic pain; and
Figure 3 shows the effect of spongosine (0.6 mg/kg p.o.) on A: blood pressure in normal rats; B: heart rate.

Examples

Example 1

Figure 1: A. Spongosine (0.624mg/kg p.o.) inhibits carrageenan (CGN) induced thermal hyperalgesia (CITH) with comparable efficacy to indomethacin (3mg/kg, po). B. Concentration-response relationship for Spongosine at 3 hrs post dosing. Carrageenan (2%, 10 microlitres) was administered into the right hind paw. A heat source was placed close to the treated and untreated hind paws, and the difference in the paw withdrawal latencies is shown. Spongosine was administered at the same time as carrageenan.

Example 2

Figure 2: Spongosine (0.624mg/kg p.o.) inhibits thermal hyperalgesia caused by chronic constriction injury of the rat sciatic nerve. Under anaesthesia the sciatic nerve was displayed

in the right leg, and four loose ligatures tied round the nerve bundle. After approximately two weeks the rats developed thermal hyperalgesia in the operated leg as judged by the difference in paw withdrawal latencies of the right and left paws. Administration of spongosine reduced the hyperalgesia as shown by the reduction in the difference between the withdrawal latencies. Spongosine was as, or more, effective than carbamazepine (CBZ, 100mg/kg s.c.)

Example 3

Figure 3: Spongosine (0.624 mg/kg p.o.) has no significant effect on blood pressure or heart rate. An implantable radiotelemetry device was placed in the abdominal cavity of 6 rats per group. The pressure catheter of the device was inserted in the abdominal aorta and two electrodes tunnelised under the skin in a lead II position (left side of abdominal cavity/right shoulder). Individual rats were placed in their own cage on a radioreceptor (DSI) for data acquisition. A: blood pressure, B; heart rate.

Spongosine is effective in inhibiting pain perception in mammals suffering from neuropathic and inflammatory pain even when administered at doses expected to give concentrations well below those known to activate adenosine receptors. At these doses it can be seen that neither the heart A1 receptors nor the vascular A2A receptors are sufficiently stimulated to cause a change in the cardiovascular status of the animals.

Spongosine can therefore be used as an anti-hyperalgesic which can be administered orally for the treatment of hyperalgesia caused as a result of neuropathy or inflammatory disease, including bowel pain, back pain, cancer pain, fibromyalgia, HIV pain, phantom limb pain, osteoarthritis, rheumatoid arthritis, post-herpes neuralgia, trigeminal neuralgia, polyneuropathy, diabetic neuropathy and post-operative pain.

Claims

1. Use of spongosine in the manufacture of a medicament for the prevention, treatment, or amelioration of hyperalgesia.

2. Use according to claim 1, wherein the hyperalgesia is neuropathic pain.

3. Use according to claim 2 for the prevention, treatment, or amelioration of bowel pain, back pain, cancer pain, HIV pain, phantom limb pain, post-operative pain, post-herpes neuralgia, or trigeminal neuralgia, or for the treatment of neuropathic pain caused by diabetic neuropathy or polyneuropathy.

4. Use according to claim 1, wherein the hyperalgesia is inflammatory pain.

5. Use according to claim 4 for the prevention, treatment, or amelioration of bowel pain, back pain, cancer pain, fibromyalgia, post-operative pain, or for the treatment of inflammatory pain caused by osteoarthritis or rheumatoid arthritis.

6. A method of preventing, treating, or ameliorating hyperalgesia which comprises administering spongosine to a subject in need of such prevention, treatment, or amelioration.

7. A method according to claim 6, wherein spongosine is administered at a dose that is one fifth to one fiftieth of the minimum dose of spongosine that gives rise to bradycardia, hypotension or tachycardia side effects in animals of the same species as the subject to which the dose is to be administered.

8. A method according to claim 7, wherein the dose is one fifth to one tenth of the minimum dose that gives rise to the side effects.

9. A method according to claim 6, wherein spongosine is administered at a dose of less than 6mg/kg.

10. A method according to claim 9, wherein spongosine is administered at a dose of at least 0.1mg/kg.

11. A method according to claim 10, wherein spongosine is administered at a dose of 0.2 to 1mg/kg.

12. A method according to any of claims 6 to 11, wherein spongosine is administered orally, parenterally, sublingually, transdermally, intrathecally, or transmucosally.

13. A method according to any of claims 6 to 12, wherein spongosine is administered at a frequency of 2 or 3 times per day.

14. A method according to any of claims 6 to 13, wherein the hyperalgesia is neuropathic pain.

15. A method according to claim 14 for the prevention, treatment, or amelioration of bowel pain, back pain, cancer pain, HIV pain, phantom limb pain, post-operative pain, post-herpes neuralgia, or trigeminal neuralgia, or for the treatment of neuropathic pain caused by diabetic neuropathy or polyneuropathy.

16. A method according to any of claims 6 to 13, wherein the hyperalgesia is inflammatory pain.

17. A method according to claim 16 for the prevention, treatment, or amelioration of bowel pain, back pain, cancer pain, fibromyalgia, post-operative pain, or for the treatment of inflammatory pain caused by osteoarthritis or rheumatoid arthritis.

18. A method according to any of claims 6 to 17, wherein the subject is a human subject.

Figure 1

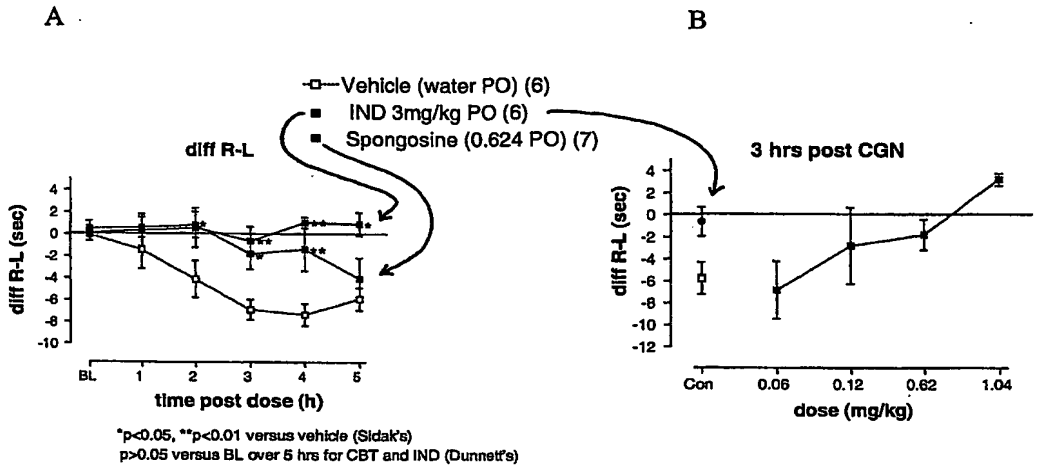

A
B
Vehicle (water PO) (6)
IND 3mg/kg PO (6)
Spongosine (0.624 PO) (7)
diff R-L
diff R-L (sec)
time post dose (h)
BL
3 hrs post CGN
Con
0.06
0.12
0.62
1.04
dose (mg/kg)
*p<0.05, **p<0.01 versus vehicle (Sidak's)
p>0.05 versus BL over 5 hrs for CBT and IND (Dunnett's)

Figure 2

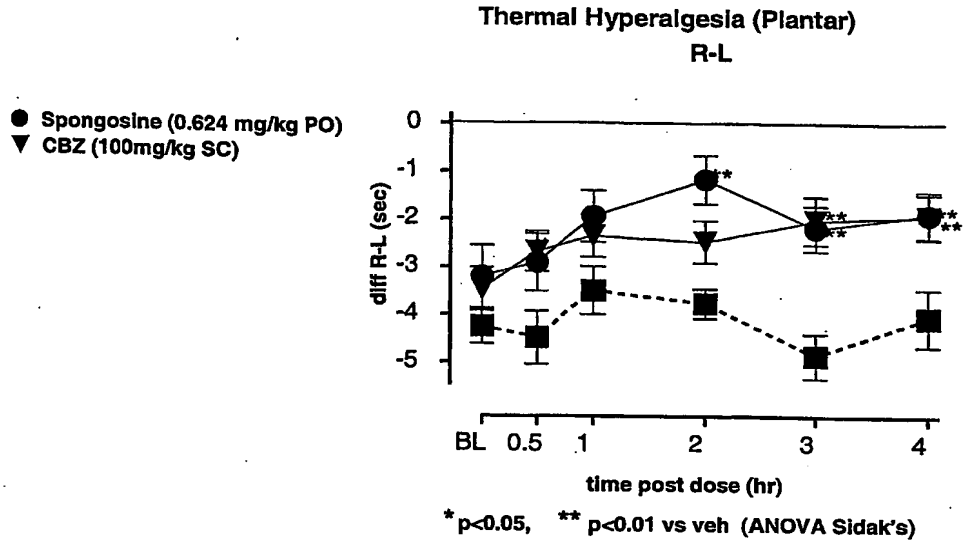
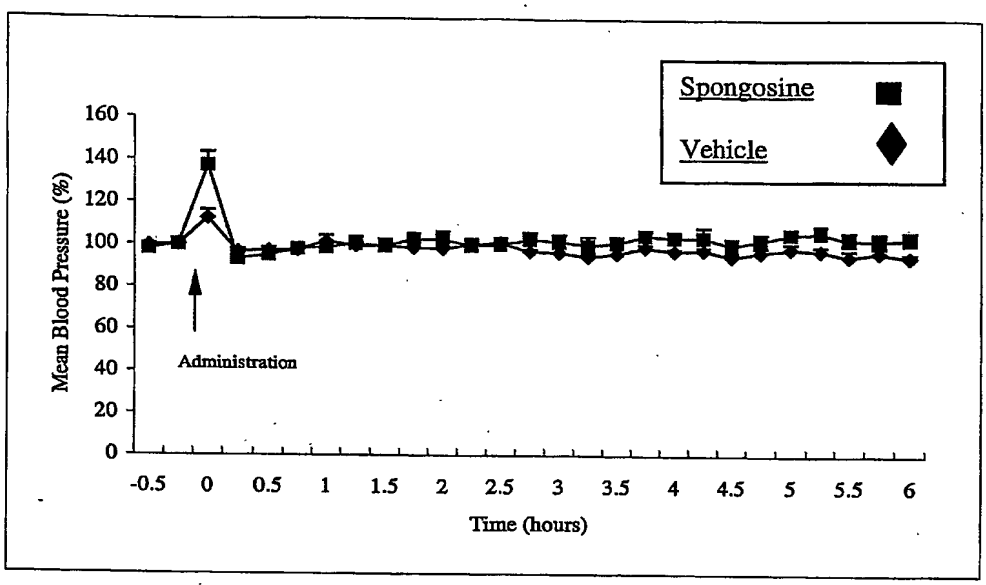
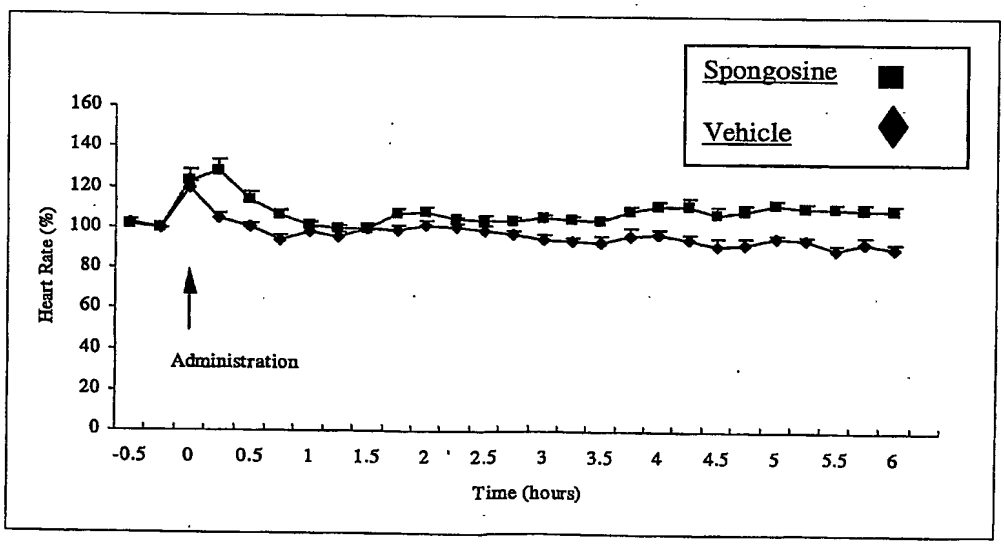

Thermal Hyperalgesia (Plantar)
R-L
Spongosine (0.624 mg/kg PO)
CBZ (100mg/kg SC)
diff R-L (sec)
0
-1
-2
-3
-4
-5
BL
0.5
1
2
3
4
time post dose (hr)
* p<0.05, ** p<0.01 vs veh (ANOVA Sidak's)

Figure 3

A


Spongosine
Vehicle
Mean Blood Pressure (%)
160
140
120
100
80
60
40
20
0
Administration
-0.5
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
5
5.5
6
Time (hours)

B.


Spongosine
Vehicle
Heart Rate (%)
160
140
120
100
80
60
40
20
0
Administration
-0.5
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
5
5.5
6
Time (hours)